



**MERCURY IN
LARGEMOUTH BASS AND SPOTTED GAR
OF THE
FLORIDA PANTHER NATIONAL WILDLIFE REFUGE**

Publication No. PCFO-EC 94-04

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**TITLE: Mercury Concentrations in Largemouth Bass and Spotted Gar of the
Florida Panther National Wildlife Refuge**

ABSTRACT: From June 21 to 25, 1990, 21 largemouth bass (*Micropterus salmoides*) and five spotted gar (*Lepisosteus oculatus*) were collected from selected locations at the Florida Panther National Wildlife Refuge, Collier County, Florida for analysis of mercury concentrations in muscle tissue. The largemouth bass were from 223 to 371 mm (8.8-14.6 in) in length. Mercury concentrations ranged from 0.19 to 0.82 mg/kg wet wt (ppm). Forty-three percent of the bass, including at least one individual in all length classes greater than or equal to 229 mm (9 in), had mercury levels that exceeded the Florida limited-consumption concentration of 0.5 ppm wet weight. The spotted gar were from 410 to 750 mm (16.1-29.5 in) in length. Mercury levels in all gar (range = 0.72 to 1.45 ppm) exceeded the limited-consumption concentration. None of the fish had concentrations of mercury in excess of Florida's no-consumption concentration (1.5 ppm). Although all the locations sampled appeared to provide environments conducive for accumulation of mercury in largemouth bass and spotted gar, bioaccumulation was greater at two sampling stations (Canal #2 west of State Highway 29 and Bullet Pond). Several fish and wildlife trust species may be at risk when utilizing the habitat areas sampled. Additional environmental contaminant studies to determine the scope, magnitude and effects of mercury contamination are recommended.

KEY WORDS: mercury, largemouth bass, spotted gar, Florida Panther National Wildlife Refuge

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INTRODUCTION

In September 1982, the Florida Game and Fresh Water Fish Commission conducted a fish survey in the Chipola River of northwest Florida to determine if the fishery was contaminated. This action was taken when it was found that a battery salvage plant located in Jackson County had released contaminated effluent into the river. Elevated levels of mercury were found in largemouth bass (*Micropterus salmoides*) collected from the Dead Lakes area. To obtain background measurements for comparison, fish were also collected from the Santa Fe River, which was thought to be relatively pristine. Results were surprising. Santa Fe River bass also contained elevated mercury levels (Bigler *et al.*, 1985). These results led to the formation of an informal interagency task force composed of personnel from the Florida Game and Fresh Water Fish Commission, the Florida Department of Environmental Regulation (now the Department of Environmental Protection), and the Florida Department of Health and Rehabilitative Services (HRS). Subsequently, a systematic statewide mercury investigation was initiated that involved the sampling of about 20 Florida lakes or streams each year. In 1988, this on-going investigation revealed elevated concentrations of mercury in largemouth bass and other species collected in the Everglades waterways of south Florida.

As a result of the State's mercury investigation, fish consumption health advisories were formulated by the HRS for largemouth bass and other species. The advisories recommend that when the average concentration of mercury in the edible portion (i.e., fillets) is between 0.5 ppm and 1.5 ppm wet weight, healthy adults should limit their consumption to no more than one meal (= 4 oz. or 113.5 gm) of fish per week. Nursing mothers, pregnant women or those who anticipate bearing children, and children under 15 years of age are advised not to eat these fish more than once a month. Fish that contain more than 1.5 ppm of mercury should not be eaten by anyone (Florida Department of Health and Rehabilitative Services, 1989). Currently, approximately one million acres of the Everglades and another one million acres of

other Florida freshwater areas have been posted with advisories (Lambou *et al.*, 1991).

U.S. Fish and Wildlife Service (Service) holdings within the State of Florida include 26 National Wildlife Refuges (NWR) encompassing more than 216,000 hectares (534,000 acres). Many federal trust species, including threatened and endangered species, migratory birds, and anadromous fishes, utilize these refuges. As refuge lands were not included in the State investigation, the Service decided to collect fish from several refuges including Florida Panther NWR (Figure 1).

The principle objectives of the NWR studies were to determine if fish had levels of contamination that might be injurious to individuals or populations of fish and wildlife species under refuge management, and sufficient to trigger issuance of human health consumption advisories for the refuges.

SITE DESCRIPTION

The U.S. Fish and Wildlife Service (Service) took a major stride towards the recovery of the Florida panther in 1989 by purchasing the initial 24,300 acres of Florida Panther National Wildlife Refuge. The purchase of the property culminated a five-year acquisition effort. In 1985, the Service published an environmental assessment entitled *Fakahatchee Strand: A Florida Panther Habitat Preservation Proposal*, which identified 88,000 acres of important panther habitat in Collier County surrounding the Fakahatchee Strand State Preserve. The document provided the legal basis for the Service to begin efforts to acquire the 30,000-acre Refuge. In the near future, an additional 5,110 acres will be added to the Refuge through a land exchange involving Department of Interior lands in Phoenix, Arizona.

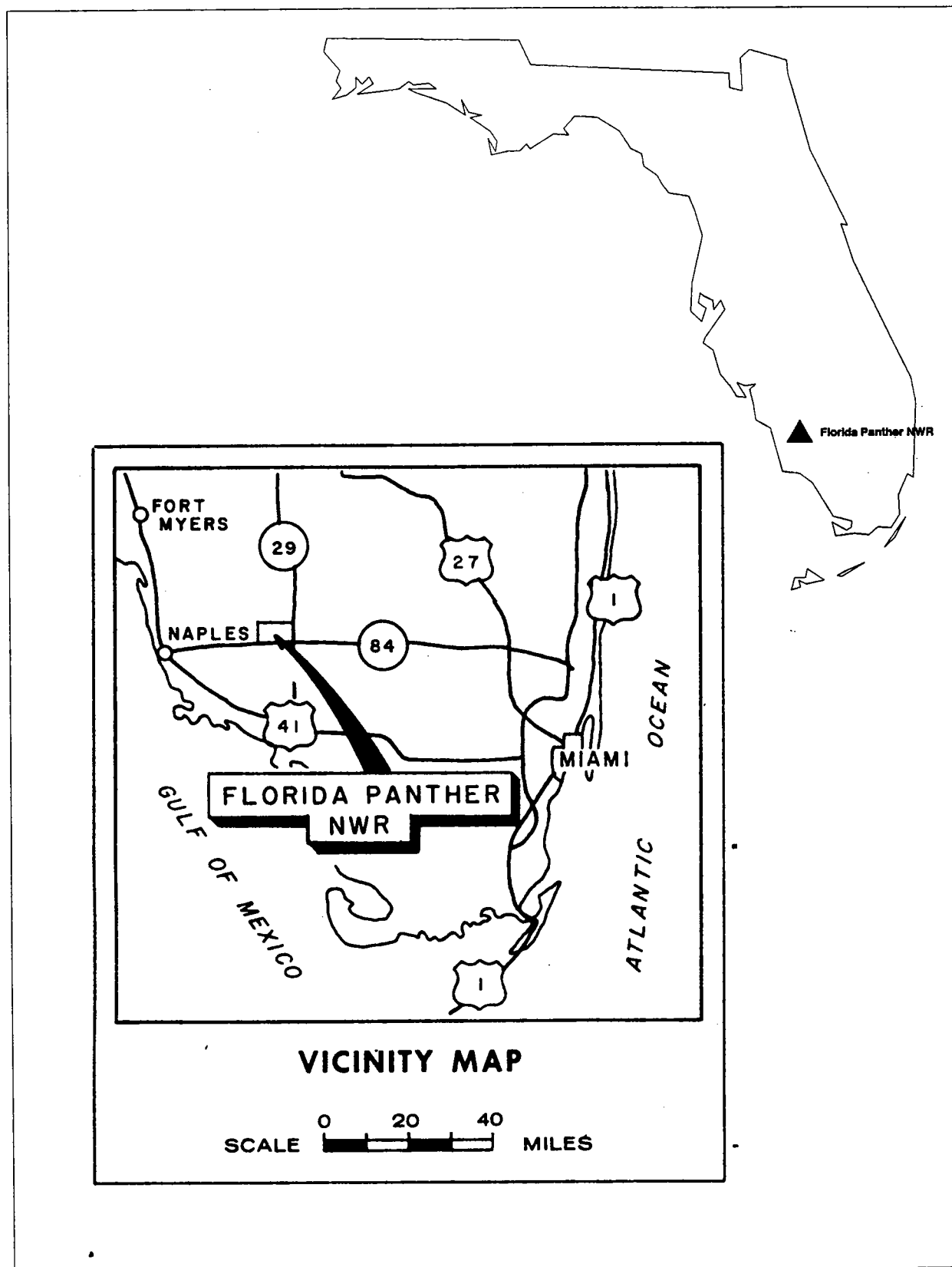


Figure 1. Location of Florida Panther National Wildlife Refuge

The northern extension of the Fakahatchee Strand, the largest strand swamp in the Big Cypress region, dominates the central portion of the Refuge. The swamp is characterized by a wetland forest of cypress trees and subtropical hardwoods. Its unique physical character creates a habitat which supports populations of rare plant species, including the largest concentration and the greatest diversity of native orchids in North America. Surrounding the strand are other wetland habitats such as wet prairies, cypress forests and mixed hardwood swamps, and upland habitats including pine flatwoods, cabbage palm forest, and hardwood hammocks.

The Refuge area has long been known to be important to the Florida panther. Radio telemetry studies being conducted by the Florida Game and Fresh Water Fish Commission continue to document extensive use of the area by the endangered cats. The Refuge forms the core of several cats' home ranges, and also functions as a travel corridor for animals traveling between the northern regions of Big Cypress National Preserve and the Fakahatchee Strand State Preserve. Several female panthers have had litters and raised kittens on the property in recent years.

The goal of the Refuge management program is to provide optimum habitat conditions for the cats. Prescribed burning will be used to maintain the native plant communities and ensure an abundance of their primary prey species, the white-tailed deer. Other programs, such as establishing food plots and wildlife clearings, will be tested. Human access to the area is limited to prevent disturbance, and the Refuge is presently not open to public activities.

Threats to the habitat and resources of this south Florida Refuge include global and regional air pollution, stormwater runoff, regional agricultural operations, water management activities, and insect control programs.

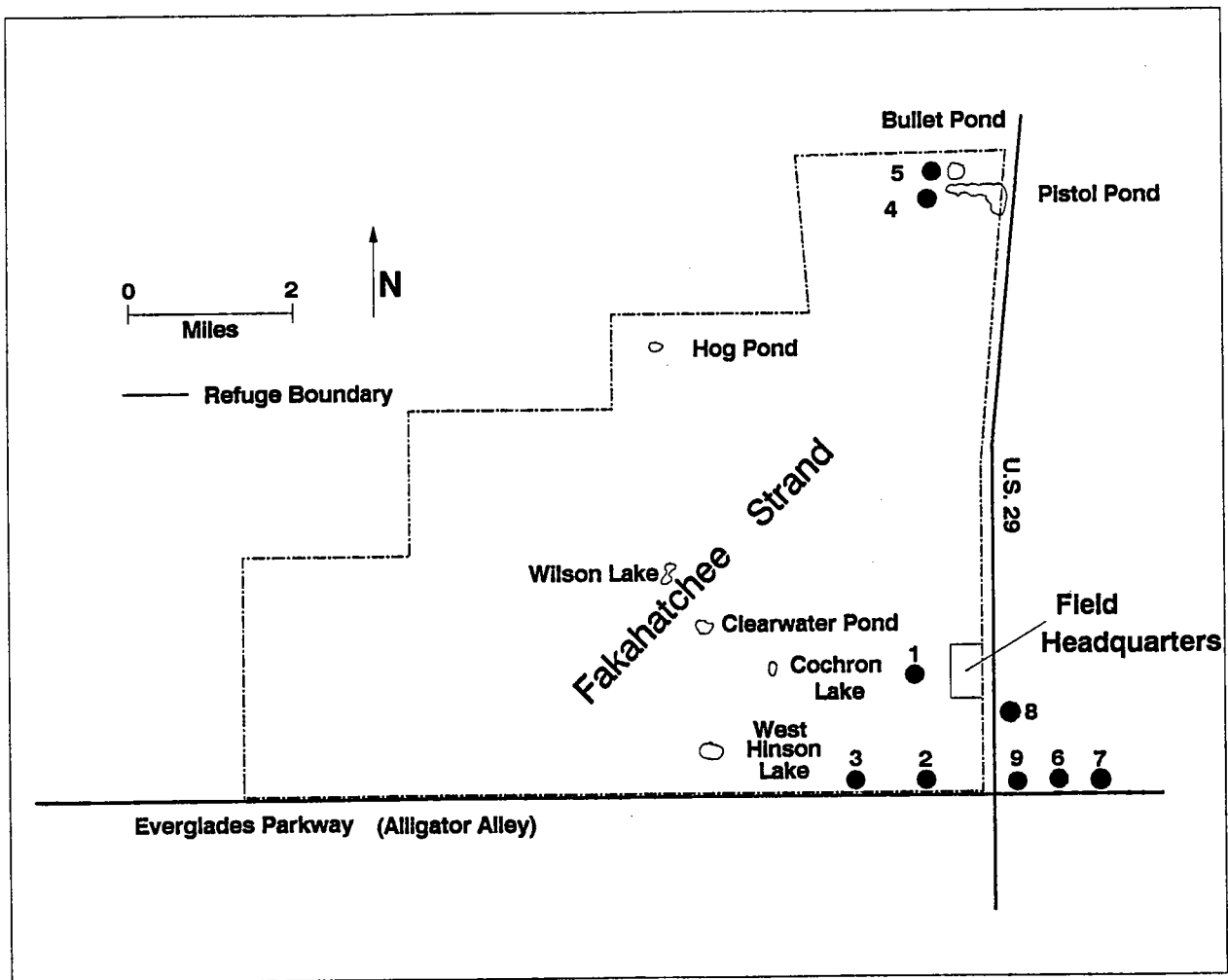
SAMPLING STATIONS

Nine stations were sampled at Florida Panther NWR (Figure 2). Three areas were ponds: Headquarters, Bullet, and Pistol. Six stations were in canals adjacent to the Refuge. The canals, which were originally borrow pits, were approximately 9 to 12 m (30-40 ft) wide. At all stations, water depth was shallow and varied between 1 and 2.4 m (3-8 ft). These stations were selected because they were the most easily accessible waterbodies for public fishing and were on or adjacent to the Refuge. Interior ponds (West Hinson Lake, Cochran Lake, Clearwater Pond, Wilson Lake, and Hog Pond) were not selected as stations because they were relatively inaccessible to the public, and almost devoid of water (due to a drought) at the time of our investigation. Appendix B contains latitudinal and longitudinal coordinates for each station.

MATERIALS AND METHODS

The primary species of interest was largemouth bass. Bass measuring eight inches in total length or longer were retained for analysis. Spotted gar (*Lepisosteus oculatus*) were collected when bass were not available. Fish were collected with gill nets or by hook and line. The latter method was used when it was not possible to launch the field boat and set gill nets in some impoundments.

Collected specimens were immediately placed on ice in clean thermal containers and were taken back to a field trailer for sample preparation. Fish samples were prepared within 4 hr of collection and stored in accordance with standard operating procedures for the collection of fish tissue samples (Appendix C). Samples were frozen within 8 hr of collection.



**Figure 2. Florida Panther National Wildlife Refuge
Fish sampling stations**

Upon returning to Panama City, samples were transferred to a storage freezer maintained at -23°C (-10°F). Samples were shipped to the analytical laboratory after approximately 120 days of freezer storage. Laboratory protocols are found in Appendix D. Appendix E contains the study data.

As mercury levels in largemouth bass have been shown to be age dependent (Lange *et al.*, 1991), sagittal otoliths were removed from each fish and forwarded to Integrated Aquatic Services, Eustis, Florida, for age determination. Aging techniques used were those described by Taubert and Tranquilli (1982).

All mercury data were transformed prior to analysis using the log transformation ($\log_{10} x$ for determining geometric mean concentrations and standard errors of the means (S.E.); $\log_{10} x + 1$ for age-normalization). Dry weight data were used for all comparisons. Single classification analysis of variance (ANOVA) and the Student-Newman-Keuls (SNK) procedure were used to detect and evaluate differences among means (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

Although we attempted to collect specimens at all nine stations, fish were obtained from only five of them. Twenty-one largemouth bass and five spotted gar were collected and analyzed for mercury in muscle tissues (i.e., fish fillets). All fillets contained some mercury, and concentrations ranged from 0.19 to 0.82 and 0.72 to 1.45 mg/kg (ppm) wet weight in bass and gar, respectively. Forty-three percent of the bass fillets ($n=9$) and all of the gar fillets contained mercury concentrations exceeding the Florida lower-level consumption advisory of 0.5 ppm mercury, wet weight. None of the tissues analyzed exceeded the upper-level consumption advisory of 1.5 ppm.

Mean mercury concentrations in bass (Table 1) were above the 0.5 ppm limit at 2 of 5 locations where bass were collected: Canal #2 west of State Highway 29 and Bullet Pond. Mean concentrations in gar were above the lower-level advisory limit at the same two stations. However, it is important for the reader to realize that sampling stations were arbitrarily selected, and that bass from other areas may contain mercury concentrations greater than those reported. In addition, at least one individual in all length classes greater than or equal to 230 mm (9 in) exceeded Florida's lower-level consumption advisory (Fig. 3). Thus, it would appear that general consumption of bass caught in those areas sampled should be limited to fish less than 230 in total length.

Table 1. Mean mercury concentrations ($\bar{x} \div$ S.E.) in fish (fillets only) from the Florida Panther National Wildlife Refuge, June 1990.

| Location | | | Concentration |
|--|-----------------------|----------|----------------|
| ID | Location ^a | <i>n</i> | (mg/kg wet wt) |
| <i>Largemouth bass (Micropterus salmoides)</i> | | | |
| 3 | Canal #2, west of 29 | 2 | 0.66(1.24) |
| 4 | Pistol Pond | 7 | 0.34(1.19) |
| 5 | Bullet Pond | 2 | 0.57(1.10) |
| 6 | Canal #2, east of 29 | 5 | 0.43(1.19) |
| 7 | Canal #3, east of 29 | 5 | 0.39(1.09) |
| <i>Spotted gar (Lepisosteus oculatus)</i> | | | |
| 3 | Canal #2, west of 29 | 3 | 0.96(1.23) |
| 5 | Bullet Pond | 2 | 0.73(1.02) |

^a See Figure 2.

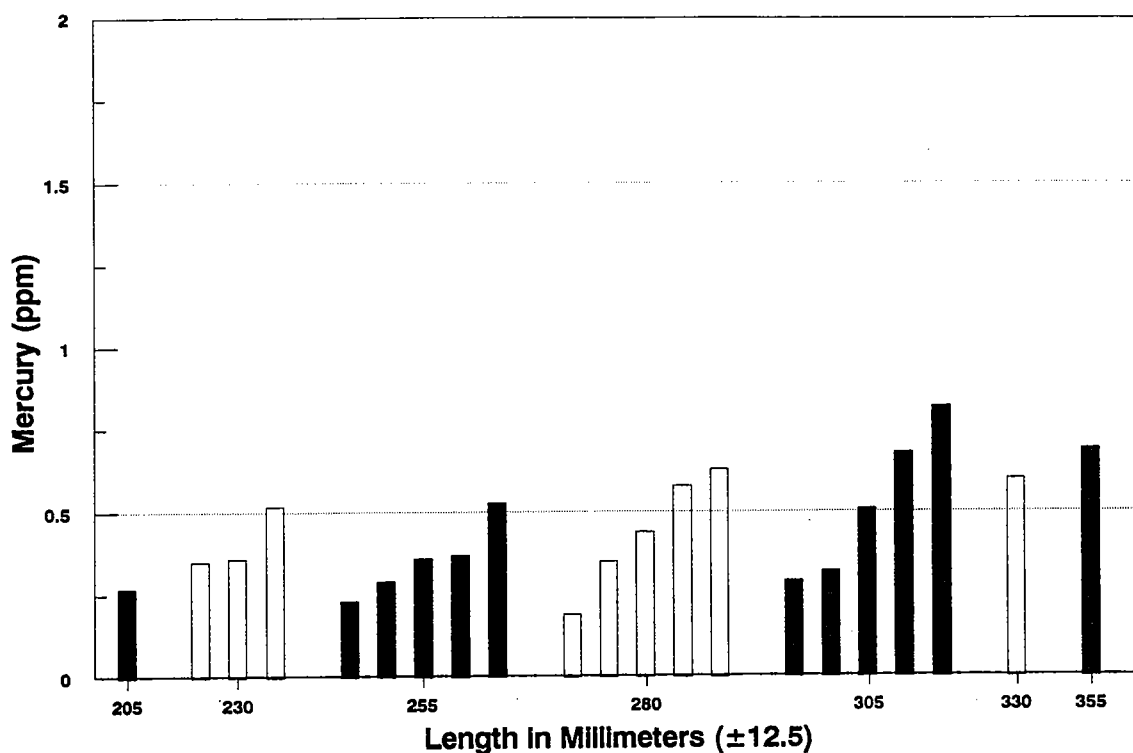


Figure 3. Mercury concentration in largemouth bass, Florida Panther National Wildlife Refuge, 1990.

The reader should also be aware that a recent bass management plan (Florida Game and Fresh Water Fish Commission, 1992) requires that bass caught in waters south and east of the Suwannee River be at least 14 inches (356 mm) in length. Thus, this requires an awareness by people fishing on the Refuge because the only fish they are allowed to take may exceed the State's lower consumption advisory.

The results of this study also suggest that some localized areas may provide an environment for fishes that results in higher tissue concentrations of mercury than in other areas. Analysis of age-normalized data revealed significant differences in mercury levels between sampling stations ($F_{(2,14)} = 6.26$; $p < 0.025$). Mercury concentrations in bass from stations 6 and 7 (Canals #2 and 3 east of State Highway 29) were significantly greater than the concentration observed at station 4 (Pistol Pond; Table 2).

There are many reasons why mercury in bass from some physical locations may be higher than that observed in others. D'Itri (1990) has pointed out that the major determinant of the amount of mercury which may be concentrated in a fish is generally the rate of the reaction which converts inorganic (metallic) mercury to organic methylmercury. Methylmercury, the predominant form found in fish fillets (Luten *et al.*, 1980), is readily bioavailable and highly toxic (Eisler 1987). The rate of methylation is dependent upon many factors including water pH, hardness, alkalinity and conductivity (D'Itri, 1990; Wiener *et al.*, 1990).

Mercury levels in bass from this Refuge were well below those reported by Armstrong (1979) to cause either chronic (e.g., loss of appetite, inability to catch food, rolling from side to side) or acute (i.e., mortality) toxicity to the fish themselves. However, concentrations in all of the fillets were above the level (0.1 ppm) which Eisler (1987) believed to be protective of sensitive species of fish-eating birds. Reproductive impairment has been noted in some avian species which regularly ingested 0.05 to 0.1 ppm in their diet (Eisler 1987).

Table 2. Age-normalized mean mercury concentrations ($\bar{x} \pm$ S.E.) in largemouth bass (*Micropterus salmoides*; fillets only) from the Florida Panther National Wildlife Refuge, June 1990.

| Location ID | Location ^a | <i>n</i> | Concentration (mg/kg wet wt) |
|----------------|-----------------------|----------|---------------------------------|
| 4 | Pistol Pond | 7 | 0.32(1.03) ^b |
| 6 | Canal #2, east of 29 | 5 | 1.01(1.16) ^c |
| 7 | Canal #3, east of 29 | 5 | 0.97(1.13) ^c |

^a See Figure 2.

^{b,c} Values with the same superscript were not significantly different from one another at $\alpha = 0.05$.

Although total mercury concentrations may be greater in whole fish than in fillets, this is usually due to high levels of inorganic mercury in the liver (Luten *et al.*, 1980). Bioaccumulation of inorganic mercury occurs at a much slower rate than methylmercury due to its relative inability to penetrate the gills and gastrointestinal tract (Olsen *et al.*, 1973). Thus, adverse effects from inorganic mercury concentrations in whole fish, as consumed by a bird or other predator, are likely to be minimal when compared to those caused by the methylmercury content of the food item.

Mink (*Mustela vison*) are reported to be one of the carnivorous species most sensitive to contaminants, including mercury, transported through the aquatic food chain (Wren, 1986). Mercury concentrations in Refuge bass were much less than 5.0 ppm, the dietary level reported to be lethal to mink by Aulerich *et al.* (1974).

CONCLUSIONS AND RECOMMENDATIONS

Mercury concentrations in fish tissue (edible fillet) from the study area sometimes exceeded State of Florida consumption advisory levels. It is possible that several species of wildlife and migratory birds feeding in waters on the Refuge may be accumulating undesirable concentrations of mercury.

The following actions are recommended:

1. Further investigation of Florida Panther National Wildlife Refuge habitat areas and biota to determine the extent and magnitude of mercury contamination;

2. Evaluation of Service trust species (endangered species and migratory birds) and their food chain organisms to determine biological effects of mercury contamination; and
3. Limitation of sport fishing for largemouth bass to catch and release; particularly in those areas where mercury concentrations are known to be high.

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APPENDIX A

THE NATURE OF MERCURY

Mercury (Hg) and its compounds have no known normal metabolic function. The presence of mercury in cells of living organisms represents contamination from either natural or anthropogenic sources, or both. Mercury contamination at the cellular level should be regarded as undesirable and potentially hazardous (Eisler 1987).

Some forms of mercury with relatively low toxicity can be transformed into forms with very high toxicity through methylation by various biotic and abiotic processes. Methyl mercury can be bioconcentrated in organisms and biomagnified through food chains, returning mercury directly to man and other upper trophic level consumers in concentrated form. Mercury has mutagenic, teratogenic and carcinogenic properties, and has caused embryocidal, cytochemical and histopathological effects. High body burdens of mercury normally encountered in some species of fish and wildlife from remote locations emphasize the complexity of natural mercury cycles and human impact on these cycles. Some scientists believe that the anthropogenic release of mercury into the environment should be curtailed because the difference between tolerable natural background levels of mercury and harmful effects in the environment is exceptionally small (Eisler 1987).

Mercury from natural sources can enter the biosphere as a gas from terrestrial and oceanic volcanic activity, in solution or in particulate form. Cinnabar (HgS) is a common mineral in hot springs deposits and a major natural source of mercury. The global cycle of mercury involves degassing of the element from the earth's crust, evaporation from natural bodies of water, atmospheric transport (mainly in the form of mercury vapor), and wet or dry deposition of mercury back onto land and water. Oceanic effluxes of mercury are tied to equatorial upwelling and

phytoplankton activity and may significantly affect the global cycling of this metal. If volatilization of mercury is proportional to primary production in the world's oceans, oceanic phytoplankton activity represents about 36 percent of the yearly mercury flow to the atmosphere (Eisler 1987).

Human activities that contribute significantly to the global input of mercury include the combustion of fossil fuels, mining and reprocessing of gold, copper, and lead, operation of chlor-alkali plants, and disposal of batteries and fluorescent lamps. The production of electrical apparatus, industrial control instruments (switches, thermometers, and barometers, etc.), laboratory appliances, anti-fouling and mildew-proofing paints, chemical formulations to control fungal diseases of seeds, bulbs, and vegetables, dental amalgams, pulp and paper, pharmaceuticals, and metallurgy and mining, is contributing, or has contributed, mercury to the environment (Eisler 1987).

Mercury burdens in sediments and other non-biological materials are estimated to have increased up to five times prehuman levels; primarily as a result of man's activities. The estimated half-time residence value for mercury is comparatively short in the atmosphere, between 6 and 90 days, but is much longer in terrestrial soils, oceanic waters, and oceanic sediments where it is estimated to remain 1,000, 2,000, and more than one million years, respectively (Eisler 1987).

An elevated concentration of mercury (usually as methyl mercury) in any biological sample is often associated with proximity to human use of mercury. The elimination of mercury point-source discharges has usually been successful in improving environmental quality. However, elevated levels of mercury in biota may persist in contaminated areas long after the source of pollution has been discontinued. It is noteworthy that some groups of organisms with consistently elevated mercury residues may have acquired these concentrations as a result of natural processes, rather than from anthropogenic activities. These groups include

older specimens of long-lived predatory fishes, marine mammals (especially seals and sea lions), and organisms living near natural mercury ore/cinnabar deposits.

Certain species of macrophytes strongly influence mercury cycling. For example, *Spartina alterniflora*, a dominant salt marsh plant in Georgia estuaries, accounted for almost half the total mercury budget in that ecosystem (Eisler 1987).

Mangrove vegetation plays a similarly important role in mercury cycling in the Florida everglades (Eisler 1987). These findings suggest that more research is needed on the role of higher plants in the mercury cycle. In aquatic ecosystems, removal of the source of anthropogenic mercury results in a slow decrease in the mercury content of sediments and biota. The rate of loss depends, in part, on the initial degree of contamination, the chemical form of the mercury and the half-life of that form, physical and chemical conditions of the system, and the hydro-dynamics of the particular aquatic ecosystem.

Methyl mercury is produced by methylation of inorganic mercury present in both freshwater and saltwater sediments, and accumulates in aquatic food chains in which the top level predators usually contain the highest concentrations (Eisler 1987). Most organomercury compounds other than methyl mercury decompose rapidly in the environment, and behave much like inorganic mercury compounds (Eisler 1987). In organisms near the top of the food chain, such as carnivorous fishes, almost all mercury accumulated is in the methylated form, primarily as a result of the consumption of prey containing methyl mercury. A strong relationship appears to exist between elevated mercury in Florida largemouth bass and low pH waters from swamp or peat drainage. A negative correlation exists in Florida for highly eutrophic (enriched) waters, where depressed mercury levels are typically found.

Methylation also occurs within the biological organisms themselves because intestinal bacteria convert mercury into methyl mercury through enzymatic

processes. However, this methylation process, as a mercury uptake source, is not as important as intake of methyl mercury via the animal's diet.

There is no known effective antidote to counteract the effects of methyl mercury poisoning on the vertebrate central nervous system (Eisler 1987). Mercury binds strongly with sulfhydryl groups and has many potential target sites during embryogenesis. Phenyl mercury and methyl mercury compounds are among the strongest inhibitors of cell division (Eisler 1987). Organomercury compounds, especially methyl mercury, cross placental barriers and can enter mammals by way of the respiratory tract, gastrointestinal tract, skin or mucous membranes (Eisler 1987). Compared with inorganic mercury compounds, organomercurials are more completely absorbed, or more soluble in organic solvents and lipids, pass more readily across biological membranes, and are slower to be excreted (Eisler 1987).

Mercury, at comparatively low concentrations, adversely affects the reproduction, growth, behavior, metabolism, blood chemistry, osmoregulation, and oxygen exchange of marine and freshwater organisms (Eisler 1987). In general, the accumulation of mercury by aquatic biota is rapid, and depuration is slow. Organomercury compounds, especially methyl mercury, have been found to be significantly more effective than inorganic mercury compounds in producing adverse effects and accumulations. Adverse effects of mercury to aquatic organisms have been documented at water concentrations of 0.88 to 5.0 ug/l. Enzyme disruption occurred in brook trout (*Salvelinus fontinalis*) embryos exposed for 17 days in solutions containing 0.88 ug/l of methyl mercury (Eisler 1987). Increased incidence of frustule abnormalities and burst thecae were documented in two species of marine algae exposed to 1.0 ug/l concentrations of Hg^{++} for 24 hours (Eisler 1987). Arrested development of sea urchin larvae occurred in a 40-hour test when the larvae were exposed to 3.0 ug/l concentrations of Hg^{++} (Eisler 1987). Decreased rate of intestinal transport of glucose, fructose, glycine, and tryptophan occurred in the murrel, *Channa punctatus*, when exposed to 3.0 ug/l

concentrations of Hg^{++} for 30 days (Eisler 1987). The blood chemistry of striped bass (*Morone saxatilis*) was altered when these fish were exposed to 5.0 ug/l concentrations of Hg^{++} for 60 days (Dawson 1982). Decreased respiration in striped bass was observed 30 days post exposure after immersion for 30 to 120 days in 5.0 ug/l concentrations of Hg^{++} (Eisler 1987).

The environmental cycle of mercury is delicately balanced and small changes in input rates, and/or the chemical forms of mercury, may result in increased methylation rates in sensitive systems. For example, the acidification of natural bodies of freshwater is statistically associated with elevated concentrations of methyl mercury in the edible tissues of predatory fishes. In chemically sensitive waterways such as poorly buffered lakes, the combined effects of acid precipitation and increased emissions of mercury to the atmosphere (with subsequent deposition) pose a serious threat to the biota if optimal biomethylation conditions are met.

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APPENDIX B

FLORIDA PANTHER NATIONAL WILDLIFE REFUGE SAMPLING STATION LOCATIONS

| Station No. | Latitude | Longitude | Township & Range |
|-------------|-------------|-------------|------------------------|
| 1 | 26°10'09" N | 81°20'54" W | Sec 30, T 49 S, R 30 E |
| 2 | 26°09'18" N | 81°21'37" W | Sec 31, T 49 S, R 30 E |
| 3 | 26°09'18" N | 81°21'54" W | Sec 36, T 49 S, R 29 E |
| 4 | 26°14'43" N | 81°20'26" W | Sec 5, T 49 S, R 30 E |
| 5 | 26°15'00" N | 81°20'26" W | Sec 5, T 49 S, R 30 E |
| 6 | 26°09'18" N | 81°20'19" W | Sec 32, T 49 S, R 30 E |
| 7 | 26°09'18" N | 81°19'53" W | Sec 32, T 49 S, R 30 E |
| 8 | 26°09'20" N | 81°28'40" W | Sec 32, T 49 S, R 30 E |
| 9 | 26°09'18" N | 81°20'33" W | Sec 32, T 49 S, R 30 E |

**STANDARD OPERATING PROCEDURES
COLLECTION OF FISH TISSUE SAMPLES**

Fish collected for chemical contaminant evaluations may be taken by electrofishing gear, monofilament gill nets, otter trawl, haul or beach seines, fish traps, trotlines, or rod and reel. However, any collecting gear should be free of chemical treatments and/or metals that could contaminate samples. This is particularly important when the entire fish (whole body analysis) will be used.

For species of special concern such as Gulf sturgeon or large broodstock striped bass, we utilize only incidental mortalities, and these should be fresh specimens.

The following is for sample dissection:

1. Wash hands thoroughly and rinse completely. Wear vinyl or latex gloves. Final rinse with distilled water.
2. Fish should be clean. It may be rinsed of debris or mud in the waters of the collection site.
3. The dissection surface (work area) should be a chemically inert substance such as a stainless steel acetone-rinsed pan, or counter. Avoid letting the dissected sample touch this surface, if possible.
4. Use previously cleaned, and acetone-rinsed, then distilled water-rinsed stainless steel dissection tools (knives, scalpels, etc.). Scales for total fish weights and sample weights should also be clean or covered with

pre-cleaned aluminum foil. Measuring devices for fish lengths, etc., should be clean, or should not come in contact with the specimen.

5. Do not let dissected samples remain exposed to the air. Exposure can dry samples and reduce the natural percentage of moisture. Prepare each dissected sample for shipping or freezing as it is dissected.
6. Samples should be placed in the smallest, pre-cleaned glass jar that will adequately hold the sample. The jars should be pre-labeled with a permanent, waterproof marking pen on the outside of the jar. Jars should also have a teflon liner inside the lid. As an alternative, acetone-rinsed, heavy-duty aluminum foil may be used to wrap the sample. After double-wrapping, place the sample (with sample identification label) inside an air-tight zip-lock bag.
7. Sample identification labels should be prepared with permanent, waterproof ink or other writing instruments that will not bleed out or wash out, and should provide the following information:
 - a. species name and common name,
 - b. type of tissue (if not whole body),
 - c. collection location,
 - d. latitude and longitude,
 - e. county and state,
 - f. weight of sample in grams,
 - g. date of collection,
 - h. sample collector's name,
 - i. total weight of fish specimen (grams),
 - j. total length and fork length of specimen (mm), and
 - k. method of collection.

8. Samples should be frozen as soon as possible. If samples contain large amounts of liquids that may expand, the lids may be set on the jars, without securing, until the sample has expanded and frozen. The lids should then be secured tightly.
9. Photographs of the specimens are desirable, as well as a written description of any external or internal lesions, tumors, etc.

APPENDIX D

LABORATORY QA/QC PROCEDURES

FOR MERCURY ANALYSIS



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Environmental Trace Substances Research Center

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Columbia, Missouri 65203
Telephone (314) 882-2151

NITRIC REFLUX DIGESTION FOR MERCURY

Approximately 0.5 g. of sample was weighed into a freshly cleaned 50 ml. round bottom flask with 24/40 ground glass neck. For waters, 10 ml. of sample were measured into the flask. Five ml. of concentrated sub-boiled HNO_3 were added and the flask was placed under a 12 inch water-cooled condenser with water running through the condenser. The heat was turned up to allow the HNO_3 to reflux no more than 1/3 the height of the columns. Samples were allowed to reflux for two hours. Then the heat was turned off and the samples allowed to cool. The condensers were rinsed with 1% v/v HCl and the flasks removed. The samples were diluted with 1% v/v HCl in a 50 ml. volumetric flask and then transferred to clean, labeled, 2 oz. flint glass bottles.



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MERCURY - COLD VAPOR ATOMIC ABSORPTION

Equipment used for Cold Vapor Atomic Absorption include: Perkin-Elmer Model 403 AA; Perkin-Elmer Model 056 recorder; Technicon Sampler I; Technicon Pump II; a glass cell with quartz windows and capillary tube for entry and exit of the mercury vapor; and a liquid-gas separator. The samples were placed in 4 ml. sample cups at least 3/4 full. The samples were mixed with hydroxylamine for preliminary reduction, then stannous chloride for reduction to the mercury vapor. The vapor was separated from the liquid and passed through the cell mounted in the light path of the burner compartment. The peaks were recorded and the peak heights measured. The standardization was done with at least 5 standards in the range of 0 to 10 ppb. The correlation coefficient was usually 0.9999 or better and must have been at least 0.999 to have been acceptable. A standard was run every 8-10 samples to check for drift in the standardization. This was usually less than 5%. Standards were preserved with 10% v/v HNO_3 , 1% v/v HCl and 0.05% w/v $\text{K}_2\text{Cr}_2\text{O}_7$. The solution concentrations were calculated and the data entered into the AA calculation program which corrected for blank, dilution, sample weight, sample volume and entered the data into the LIMS system for report generation.

APPENDIX E

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Florida Panther NWR - Mercury/Fish Study, 1990

| #90-104A | Station Location | Species | Total length mm | Total Length inches | Total Weight gm | Total Weight oz | Hg conc. dry wt mg/kg (ppm) | Sample % moisture | Hg conc. wet wt mg/kg (ppm) | Sex | Age otolith |
|----------|---------------------|-----------------|-----------------|---------------------|-----------------|-----------------|-----------------------------|-------------------|-----------------------------|-----|-------------|
| FP1 | Pond behind HDQTRS | NONE TAKEN | | | | | | | | | |
| FP2 | Canal#1, west of 29 | NONE TAKEN | | | | | | | | | |
| FP3-1 | Canal#2, west of 29 | LARGEMOUTH BASS | 327 | 12 | 530 | 18 | 3.61 | 77.4 | 0.82 | ND | 3 |
| FP3-2 | Canal#2, west of 29 | SPOTTED GAR | 750 | 29 | 1855 | 65 | 6.48 | 77.7 | 1.45 | F | 4 |
| FP3-3 | Canal#2, west of 29 | SPOTTED GAR | 459 | 18 | 400 | 14 | 3.56 | 79.5 | 0.73 | F | 4 |
| FP3-4 | Canal#2, west of 29 | SPOTTED GAR | 465 | 18 | 410 | 14 | 3.98 | 79.2 | 0.83 | F | ND |
| FP3-5 | Canal#2, west of 29 | LARGEMOUTH BASS | 275 | 10 | 275 | 9 | 2.33 | 77.4 | 0.53 | F | 1 |
| FP4-1 | Pistol Pond | LARGEMOUTH BASS | 316 | 12 | 327 | 11 | 1.4 | 79 | 0.29 | F | 4 |
| FP4-2 | Pistol Pond | LARGEMOUTH BASS | 277 | 10 | 190 | 6 | 1.4 | 79.1 | 0.29 | M | 3 |
| FP4-3 | Pistol Pond | LARGEMOUTH BASS | 260 | 10 | 160 | 5 | 1.1 | 79.1 | 0.23 | M | 3 |
| FP4-4 | Pistol Pond | LARGEMOUTH BASS | 290 | 11 | 275 | 9 | 0.95 | 79.6 | 0.19 | M | 3 |
| FP4-5 | Pistol Pond | LARGEMOUTH BASS | 289 | 11 | 250 | 8 | 1.7 | 79.3 | 0.35 | M | 4 |
| FP4-6 | Pistol Pond | LARGEMOUTH BASS | 294 | 11 | 260 | 9 | 2.76 | 78.9 | 0.58 | F | 4 |
| FP4-7 | Pistol Pond | LARGEMOUTH BASS | 371 | 14 | 430 | 15 | 3.99 | 82.8 | 0.69 | F | 4 |
| FP5-1 | Bullet Pond | LARGEMOUTH BASS | 283 | 11 | 230 | 8 | 2.88 | 78.1 | 0.63 | M | 4 |
| FP5-2 | Bullet Pond | LARGEMOUTH BASS | 250 | 9 | 153 | 5 | 2.42 | 78.4 | 0.52 | F | 2 |
| FP5-3 | Bullet Pond | SPOTTED GAR | 417 | 16 | 270 | 9 | 3.77 | 80.1 | 0.75 | M | 3 |
| FP5-4 | Bullet Pond | SPOTTED GAR | 410 | 16 | 260 | 9 | 3.58 | 79.9 | 0.72 | F | 3 |
| FP6-1 | Canal#2, east of 29 | LARGEMOUTH BASS | 256 | 10 | 175 | 6 | 1.63 | 77.3 | 0.37 | F | 1 |
| FP6-2 | Canal#2, east of 29 | LARGEMOUTH BASS | 263 | 10 | 185 | 6 | 1.71 | 79 | 0.36 | M | 1 |
| FP6-3 | Canal#2, east of 29 | LARGEMOUTH BASS | 342 | 13 | 500 | 17 | 2.78 | 78.5 | 0.6 | F | 4 |
| FP6-4 | Canal#2, east of 29 | LARGEMOUTH BASS | 305 | 12 | 340 | 11 | 3.3 | 79.4 | 0.68 | M | 4 |
| FP6-5 | Canal#2, east of 29 | LARGEMOUTH BASS | 223 | 8 | 120 | 4 | 1.3 | 78.9 | 0.27 | M | 0 |
| FP7-1 | Canal#3, east of 29 | LARGEMOUTH BASS | 312 | 12 | 380 | 13 | 2.38 | 78.6 | 0.51 | F | 3 |
| FP7-2 | Canal#3, east of 29 | LARGEMOUTH BASS | 330 | 12 | 415 | 14 | 1.5 | 78.8 | 0.32 | M | 2 |
| FP7-3 | Canal#3, east of 29 | LARGEMOUTH BASS | 285 | 11 | 295 | 10 | 2.09 | 78.9 | 0.44 | F | 2 |
| FP7-4 | Canal#3, east of 29 | LARGEMOUTH BASS | 229 | 9 | 145 | 5 | 1.7 | 78.7 | 0.36 | F | 1 |
| FP7-5 | Canal#3, east of 29 | LARGEMOUTH BASS | 237 | 9 | 145 | 5 | 1.61 | 78.2 | 0.35 | F | 1 |
| FP8 | Canal on 29 | NONE TAKEN | | | | | | | | | |
| FP9 | Canal#1, east on AA | NONE TAKEN | | | | | | | | | |

Note: ND = not determined.